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Agriculture Development Fund

FINAL REPORT

19990251

THE EFFECTS OF PROPHYLACTIC ANTIBIOTICS ON THE DEVELOPMENT OF ANTIMICROBIAL-RESISTANT BACTERIA IN FEEDLOT CATTLE

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The Effect of Prophylactic Antibiotics on the Development of Antimicrobial-Resistant Bacteria in Feedlot Cattle.

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Principle Investigator:
Dr. John Campbell
Dept. of Large Animal Clinical Sciences
Western College of Veterinary Medicine

ABSTRACT:

Two hundred and eighty-eight auction market derived weaned beef calves were placed in small pens (12 head each) at the University of Saskatchewan feedlot. Each pen was randomized to receive one of three treatments:

1) 2g/head/day of oxytetracycline in the starter ration
2) 20 mg/kg body weight of long-acting oxytetracyline administered subcutaneously on day 1 of the trial period
3) control pens which received no antimicrobials for prophylaxis

Fecal samples were collected from each calf at day 1, day 7, day15, day 35 and just prior to slaughter. Three isolates of fecal Escherichia coli were cultured from each of these samples and were evaluated for Minimum Inhibitory Concentrations for 8 different antimicrobials. None of the isolates collected demonstrated resistance to two important human antibiotics Enrofloxacin or Gentamicin.

Eighty-five percent of the isolates collected at arrival showed no evidence of resistance to any of the antimicrobials evaluated. The use of oxytetracycline in the starter ration had a significant effect on the proportion of isolates resistant to various antibiotics at day 7, day 15 and day 35. However, at pre-slaughter there was no longer a treatment effect. The use of long-acting injectable oxytetracyline had no significant effects on the proportion of resistant isolates at any time. The use of antibiotics to treat a significant proportion of foot rot cases near the end of the feeding period may have had a significant effect on the proportion of antibiotic resistant fecal E. coli isolates at the pre-slaughter time period.

EXECUTIVE SUMMARY:

The objectives of this study were to:

1) Determine the prevalence of fecal bacteria resistant to various antibiotics in auction market derived weaned calves upon arrival to the feedlot.
2) Determine the effect of prophylactic antibiotic usage on the development of fecal bacteria resistant to various antibiotics.
3) Determine the persistence of antibiotic-resistant fecal bacteria and evaluate the potential risk of their presence at slaughter

Two hundred and eighty-eight auction market derived weaned beef calves were placed in the University of Saskatchewan research feedlot. After processing, each pen of 12 calves were assigned to one of three treatments: Oxytetracycline in the starter ration for 14 days at the level of 2g/head/day; long acting injectable oxytetracyline given subcutaneously at the dose of 20 mg/kg of body weight; and a control group which received no prophylactic or metaphylactic antimicrobials. Fecal samples were collected from each calf prior to treatment administration (day 1), day 7, day 15, day 35, and immediately prior to slaughter (average 208 days on feed). Fecal samples were also collected at day 70, day 100 and day 150. These samples will be evaluated at a later date. Three isolates of Escherichia coli were cultured from each of the calves at each collection period and the Minimum Inhibitory Concentration of 8 antibiotics was ascertained for each isolate. These MIC's were used to determine whether or not the bacterial isolate was susceptible or resistant to that particular antibiotic. The antibiotics that were evaluated were tetracycline, ampicillin, florfenicol, gentamicin, enrofloxacin, trimethoprim-sulfadoxine, trimethoprim, and sulphadoxazole. None of the E. coli isolates demonstrated resistance to gentamicin or enrofloxacin which are two important antibiotics in human medicine that are not approved for use in the bovine. Eighty-five percent of the E.coli isolates recovered on arrival from the calves showed no resistance to any of the antibiotics being evaluated. However, 2.2% of the isolates exhibited resistance to 3 or more antimicrobials which demonstrated that some of the isolates were exhibiting multi-drug resistance.

Significant differences between treatment groups were seen at arrival for Florfenicol; at day 7 for Florfenicol, Sulphamethoxazole, Tetracycline and Trimethoprim-sulfa; at day 15 for Sulphamethoxazole, Tetracycline and Trimethoprim-sulfa , and at day 35 for Sulphamethoxazole and Tetracycline. There were no significant differences in proportion of resistant isolates between treatment groups prior to slaughter for any of the antibiotics tested.

Conclusions

(1) Weaned auction market derived calves have a low prevalence of antibiotic resistant isolates of E. coli in their feces on arrival to the feedlot. However, 2.2% of these fecal E. coli isolates collected at arrival do show multi-drug resistance to 3 or more antibiotics.

(2) There is no evidence of any fecal E. coli resistance to Enrofloxacin or Gentamicin in the population of weaned auction market calves sampled. These drugs are important antibiotics in human medicine but are not approved for use in the bovine in Canada.

(3) The administration of oxytetracycline in the starter ration for the first 14 days at the level of 2g/head/day results in a significant increase in the proportion of resistant organisms. However, this treatment effect is no longer evident at slaughter.

(4) The use of long-acting oxytetracycline as a metaphylactic drug on arrival to the feedlot does not result in significant increases in the proportion of antibiotic resistant fecal E. coli isolates.

(5) The use of antibiotics to treat foot-rot in feedlot cattle which tends to occur late in the feeding period may result in an increase in the prevalence of fecal E. coli isolates that are resistant to antibiotics. Further research is necessary to validate this conclusion.

Recommendations:

Metaphylaxis or the use of injectable long acting antimicrobials on arrival to feedlot cattle to prevent bovine respiratory disease is supported by this research. Although this study did not show a significant decrease in respiratory disease, the trend was definitely in that direction. As well, metaphylaxis did not have any significant effect on the development of long-term antibiotic resistance in these animals.

Prevention of foot rot and other infectious diseases that occur late in the feeding period should be given priority in order to minimize the use of antibiotics at this critical time period.

BACKGROUND:

Multi-drug resistant pathogens have become a significant problem in human infectious diseases. Many bacteria that cause human disease have developed resistance to antibiotics commonly used for treatment. Multi-drug resistant bacteria have also become evident in various livestock populations. The emergence of Salmonella typhimurium DT104 in the cattle populations of the United Kingdom and North America throughout the last decade is just one example. The potential of the use of antimicrobials in livestock contributing to the antimicrobial resistance seen in the human population has received a great deal of attention in the last few years. The development of bacteria resistant to antibiotics could limit the producer's ability to deal with infectious disease in the feedlot and the potential of resistant strains of bacteria entering the food chain is obviously an important food-safety issue. European countries have already banned the use of antibiotics in livestock feed for growth promotion purposes because of the perceived risk of developing antibiotic resistant bacteria that may impact on human health. There has been some circumstantial evidence of the use of antibiotics in livestock contributing to antimicrobial resistance in people. Much of this evidence has focused on the use avoparcin as a growth promotant in poultry feed and the development of vancomycin resistant enterococci. Many organizations in both the veterinary and human medical communities have attempted to develop guidelines regarding the prudent use of antimicrobials. However, most of these guidelines are very general in nature and there are few specific recommendations because of a paucity of scientific information on the effects of utilizing antimicrobials in livestock on antimicrobial resistance.

In the cattle feeding industry in North America, antibiotics are commonly used on arrival to prevent and control bovine respiratory disease. Bovine respiratory disease (BRD) is often touted as the most economically important infectious disease of calves in North American feedlots. Despite available vaccines, the high incidence of this disease in weaned beef calves dictates that many producers rely on the use of antimicrobials to limit the economic impact of BRD. A high percentage of the calves which are purchased from auction markets may already be incubating respiratory disease or be clinically sick when they arrive at the feedlot. The use of antibiotics in the starter ration or the use of injectable antibiotics on arrival (metaphylaxis) has become a very common and economically advantageous technique employed by feedlots to control bovine respiratory disease. The long-term effect of the use of these antibiotics on the development of antimicrobial resistance is unknown.

The objectives of this study were to:

4) Determine the prevalence of fecal bacteria resistant to various antibiotics in auction market derived weaned calves upon arrival to the feedlot.
5) Determine the effect of prophylactic antibiotic usage on the development of fecal bacteria resistant to various antibiotics.
6) Determine the persistence of antibiotic-resistant fecal bacteria and evaluate the potential risk of their presence at slaughter.

MATERIALS AND METHODS

Trial Facilities

The trial was conducted at the small-pen research feedlot at the University of Saskatchewan in Saskatoon. The calves were housed in open-air pens with dirt floors and a central alley as is representative of the larger feedlots in Western Canada. The pens are 41 feet wide by 78 feet deep with a 24 foot feed bunk facing a central alley. Water bowls are shared between two pens. The pens are surrounded on three sides by 20% porosity fencing. There are 28 pens available which each have a capacity of approximately 15 animals.

Trial Animals

In the fall of 2000, 288 auction-market derived, 200-275 kg, Charolais cross steers were purchased in Saskatchewan and brought to the feedlot facility at the University of Saskatchewan. The calves were allowed to adjust to the feed bunks for several days while the entire group was accumulated. All calves were processed on day 0 of the trial. The processing protocol included vaccination with a multivalent Clostridial vaccine with *Hemophilus somnus* [(Fermicon 7-Somnugen™, Pfizer Canada Inc, London, Ontario) or (Ultrabac™7/Somnubac™, Pfizer Canada Inc, London, Ontario)], vaccination with a modified live vaccine for Infectious Bovine Rhinotracheitis, and Parainfluenza 3 (Bovishield™ IBR-PI_3, Pfizer Canada Inc, London, Ontario), topical parasiticidal treatment (Ivomec® Pour-On, Merial Canada Inc., Baie D'Urfe, Quebec), eartags, and a progesterone-estradiol benzoate hormonal implant (Synovex®-S, Ayerst, Veterinary Laboratories, Guelph, Ontario). A routine open castration was performed on one calf with a retained testicle.

Experimental Design

Twelve steers were randomly blocked by weight into each pen. In total, there were 24 pens used. The pens, in pairs, were randomly assigned one of three treatments: no treatment (Control), a medicated starter ration (Feed), or metaphylactic injectable long-acting antibiotic treatment at day 0 (Injectable). Randomization was performed by using a random number table at the time of processing. Oxytetracycline (Terramycin*-50 Premix, Pfizer), which has 110g/kg active ingredient, was fed at a level of 2g/head/day for 14 days for the Feed group only. This is a common dose used in feedlots in western Canada. The injectable metaphylactic treatment was a single subcutaneous injection of long-acting oxytetracycline (Liquamycin* LA-200*, Pfizer Canada Inc, London, Ontario) during processing at the label dose of 20mg/kg of body weight of the steer.

Throughout the trial, all attempts were made to ensure that there was no cross contamination between treatment groups. Pens of animals that shared water bowls were in the same treatment group in order to prevent cross-contamination between treatment groups. Control animals in a preset order, were always moved through the handling

system first on sampling days. The feedlot workers and the laboratory staff were blinded as to the treatment groups as well as the aims of the study.

Fecal samples were collected on arrival (day 1), day 7, day 15, day 35, day 70, day 100 day 150 and just prior to slaughter. Only samples from day 1, day 7, day 14, day 35 and pre-slaughter have been evaluated at the time of this report. Samples from day 70, 100 and 150 will be evaluated in the near future. The time of the pre-slaughter sample varied within the steers based on when the cattle reached 8 mm of backfat or when they reached a maximum finish weight of 750 kg. The pre-slaughter fecal sample was collected 24 hours prior to leaving the feedlot. At each sampling, each steer had a fresh fecal sample collected from the rectum. A clean, new, plastic obstetrical glove was used for each animal. The samples were placed into clean styrofoam cups with lids and were taken to the microbiology laboratory within two hours.

Animal Health

All sick animals were treated according to defined protocols. Animals that were identified as sick, with a rectal temperature over 40.5°C with no other identifiable cause of disease were considered to have bovine respiratory disease complex (BRD). Initial BRD cases received Tilmicosin (Micotil®, Provel,) at the label dose of 10 mg/kg subcutaneously and were returned to their home pen. Relapses were considered as cases identified for re-treatment of the same disease process within a two week period. Relapses were treated with a single subcutaneous injection of Florfenicol (Nuflor®, Schering-Plough Animal Health, Pointe Claire, Quebec) and returned to the home pen. Cases which relapsed for a second time were given trimethoprim/sulfadoxine for 3 consecutive days(Trivetrin™ Injection, Schering-Plough Animal Health, Pointe Claire, Quebec). Once any treatment was administered, the animal was returned to it's home pen immediately.

The other common condition where individual animal antimicrobial treatments were required was infectious pododermatitis, or footrot. The case definition for footrot was a lame animal with a swollen foot and no other obvious causes of lameness (such as joint swelling). The treatment protocol recommended daily use of ceftiofur sodium (Excenel® Sterile Powder, Pharmacia Animal Health, Orangeville, Ontario) for 2 to 3 days. Procaine Penicillin G was also used for the treatment of footrot [(Depocillin®, Intervet Canada Ltd., Whitby, Ontario or Ethacillin, Pfizer Canada Inc., London, Ontario)] at an extra-label dose of 500,000 IU per 50 kg of body weight of the animal. Both of these antimicrobials were generally given for 3 consecutive days to footrot cases.

Laboratory Analysis

All laboratory procedures were carried out according to NCCLS standards. The fresh feces were cultured overnight on MacConkey's agar and three individual strains were isolated with no further passage in vitro that might contribute to loss of plasmids coding for antimicrobial resistance. Three strains from each animal were randomly chosen and these strains were subcultured in litmus milk and stored at –70°C until a large group of antimicrobial sensitivities could be tested together. Primary cultures were also stored at –70°C. The subcultures were thawed and immediately cultured on blood agar. From each blood agar plate, E. coli were inoculated into Phosphate Buffer Sterile (PBS)

to make standard solutions of 0.5 MacFarland. This solution was delivered onto Mueller-Hinton agar using the replicator technique. Each Mueller-Hinton agar plate incorporated an antimicrobial agent at a specific dilution. Each strain of E. coli was tested at multiple dilutions of each antimicrobial to determine the Minimal Inhibitory Concentration (MIC) of that antimicobial. The MICs of eight antibiotics were determined using this agar dilution method. The antibiotics tested were ampicillin, tetracycline, florfenicol, sulphamethoxazole, gentamycin, enrofloxacin, trimethoprim and trimethoprim/sulfadoxine. The Mueller-Hinton plates were cultured at 37°C and antimicrobial sensitivities were read between 18 and 24 hours. A control strain of bacteria ATCC 25922 was included with each plate.

Statistical Analysis:

The MIC cut-off values that were used to determine various antimicrobial resistance patterns are shown in Table 1. Those isolates that had MIC values fall within the range of intermediate or susceptible were considered susceptible. MIC values that were greater than the cut-off value were considered resistant strains to that particular antimicrobial. All data were entered into a computer database and descriptive statistics were generated. Analyses of isolation rates of resistance were all calculated at the pen level. The proportion of resistant isolates were calculated by dividing the number of resistant isolates from the pen by the total number of isolates recovered from the pen. Analysis of variance was used to compare the isolation rates at the pen level between treatments. The Bonferroni test was used as the post-test comparison of means if the initial F statistic was significant in the analysis of variance.

RESULTS

Morbidity Rates

The overall morbidity rate for bovine respiratory disease in this study was 17% (51/288). The control group had a morbidity rate of 22.9% (22/96), the feed group had a morbidity rate of 16.7% (16/96) and the injectable group had a morbidity rate of 13.5% (13/96). These rates were not significantly different when compared across treatment groups at the pen level (p=.2687). Significant numbers of animals also received antimicrobials as a result of treatment for infectious pododermatitis (foot rot). The Control, Feed and Injectable groups had 27.1%, 20.8% and 21.9% of the calves treated for foot rot respectively. These means were not significantly different when compared across treatment groups at the pen level (p=.4957). The average number of days on feed at the time of treatment for foot rot was 148 days. Four of the 66 total foot rot treatments (6.1%) occurred before 125 days on feed.

Antimicrobial Resistance Patterns of E. coli Isolates

None of the E. coli isolates cultured displayed resistance to Enrofloxacin or Gentamycin. Because of the high degree of correlation between trimethoprim/sulfadoxine resistance and trimethoprim resistance, only the former is utilized in the analysis of results. The proportion of isolates at arrival that are resistant to none, one, two or three or more antbiotics are displayed in Table 2. The prevalence of resistant bacteria isolates to five different antimicrobials (Tetracycline, Florfenicol, Ampicillin, Sulphamethoxazole, and Trimethoprim sulfa) over each sampling period is displayed in Table 3. Figures 1-5 display graphical summaries of the proportion of resistant isolates to each of these antimicrobial drugs.

Significant differences between treatment groups were seen at arrival for Florfenicol; at day 7 for Florfenicol, Sulphamethoxazole, Tetracycline and Trimethoprim-sulfa; at day 15 for Sulphamethoxazole, Tetracycline and Trimethoprim-sulfa , and at day 35 for Sulphamethoxazole and Tetracycline. There were no significant differences in proportion of resistant isolates between treatment groups prior to slaughter for any of the antibiotics tested.

DISCUSSION

There were no significant effects of either metaphylaxis or prophylactic feed antimicrobials on the incidence of bovine respiratory disease. Although there was a trend towards lower treatments in the injectable group (metaphylaxis) and in the prophylactic oral antimicrobial group, these trends were not statistically significant because of the small size of the study. Small pen studies are often typified by having less respiratory disease than trials conducted in commercial sized pens of 200-300 animals because there is less mixing of animals from various sources in the small pens. This will often result in less respiratory disease and will result in a trial with less power.

It is important to emphasize the fact there were no isolates of E. coli cultured that displayed any resistance to either Enrofloxacin or Gentamicin. Both of these antimicrobials are important drugs used in human medicine and there was absolutely no evidence of resistance against these drugs in the cattle population sampled. There was however some evidence of antimicrobial resistance of E.coli at the time of arrival of these calves to the feedlot. 15.4% of the strains isolated at arrival were resistant to one or more antibiotics. Evidence of multi-strain resistance (resistant to 3 or more antimicrobials) was seen in 2.2% of isolates. These calves were all recently weaned from cow-calf farms and sold through the auction market system in Saskatchewan. It is possible there were some cases of bovine respiratory system which were treated prior to entering the auction market system which might explain some of the resistance seen at this time. Another possible explanation is that this is the normal amount of "background" resistance seen in fecal isolates of E. coli in weaned calves in Saskatchewan.

Figures 1 to 5 show a similar pattern for resistant isolates for all antimicrobial drugs tested. In most cases there is a significant peak in the isolation of resistant organisms shortly after the administration of antibiotics at arrival. Treatment differences are significant at a number of time intervals for a number of drugs (see Table 3), however, in virtually all cases the group which received oxytetracyline in the feed has a significantly higher proportion of resistant E. coli organisms isolated from their feces. As well, the group that received long-acting injectable oxytetracyline is not significantly different from the control group. It is especially important to note that there were no significant differences in the proportion of antibiotic-resistant E. coli from the pre-slaughter isolates. This would indicate that the effect of giving oxytetracyline in the feed for the first 14 days of the feeding period or using injectable long acting oxytetracyline as a metphylactic treatment on arrival did not affect the proportion of resistant organisms in the animal's feces at slaughter.

It does appear that the proportion of resistant organisms does increase overall in the pre-slaughter samples despite dropping off by day 35. However, this increase in the proportion of resistant organisms occurs in all treatment groups and there is no significant treatment effect at this time. A possible explanation for this rise in pre-slaughter resistance is that the large proportion of foot rot cases that occurred late in the feeding period contributed to the resistance in the population of pre-slaughter isolates of E.coli. The foot rot cases were distributed evenly across treatment groups and generally occurred in the last 75 days of the feeding period. The late administration of antibiotics to a significant proportion of these cattle late in the feeding period is certainly a plausible

hypothesis for why there is a rise in the proportion of resistance isolates. It is also possible that despite all the precautions that were taken, fecal cross-contamination occurred between treatment groups late in the feeding period, which eliminated any treatment differences that existed.

CONCLUSIONS

(1) Weaned auction market derived calves have a low prevalence of antibiotic resistant isolates of E. coli in their feces on arrival to the feedlot. However, 2.2% of these fecal E. coli isolates collected at arrival do show multi-drug resistance to 3 or more antibiotics.

(2) There is no evidence of any fecal E. coli resistance to Enrofloxacin or Gentamicin in the population of weaned auction market calves sampled. These drugs are important antibiotics in human medicine but are not approved for use in the bovine in Canada.

(3) The administration of oxytetracycline in the starter ration for the first 14 days at the level of 2g/head/day results in a significant increase in the proportion of resistant organisms. However, this treatment effect is no longer evident at slaughter.

(4) The use of long-acting oxytetracycline as a metaphylactic drug on arrival to the feedlot does not result in significant increases in the proportion of antibiotic resistant fecal E. coli isolates.

(5) The use of antibiotics to treat foot-rot in feedlot cattle which tends to occur late in the feeding period may result in an increase in the prevalence of fecal E. coli isolates that are resistant to antibiotics. Further research is necessary to validate this conclusion.

Indications for future research

There is a great deal of research that could be initiated which is related to the findings of this research project. There is very little information on what constitutes the prudent use of antimicrobial drugs at the cow-calf farm. An epidemiologic study examining the effects of antimicrobial use on cow-calf farms on the development of antimicrobial resistance would be an important contribution to the scientific literature. More detailed evaluation of the data from this project will continue as part of a PhD program for a PhD student in the department of Large Animal Clinical Sciences at the Western College of Veterinary Medicine. The effect of treating for foot rot late in the feeding period on antimicrobial resistance and the effectiveness of foot rot vaccines to limit this antimicrobial use is of special interest.

Information of benefit to producers

The results of this project support the use of antibiotics, as a tool, in the management of bovine respiratory disease in the feedlot. In the face of increasing public pressure and scrutiny, producers will be forced to justify the use of antibiotics, especially when used for prophylactic reasons. The results of this project would especially support the use of long-acting injectable antibiotics as a metaphylaxis treatment as it does not significantly increase the proportion of isolates of fecal E. coli that are resistant to various antibiotics at any time during the feeding period.

Project activities

The results of this project will be presented at a number of scientific conferences specifically to bovine practitioners. Preliminary results are to be presented at the Academy of Veterinary Consultants in Denver, Colorado in November 2001. The support of the Saskatchewan Agriculture Development Fund will be gratefully acknowledged in all presentations.

Table 1: Cutoff values for Mean Inhibitory Concentrations to Categorize Isolates as Resistant, Intermediate or Susceptible

Antibiotic	Susceptible	Intermediate	Resistant
Gentamicin	<=4	8	>=16
Enrofloxacin	0.25	0.5-1	>=2
Trimethoprim-sulfa	<=2/38	N/A	>=4/76
Trimethoprim	<=8	N/A	>=16
Ampicillin	<=8	16	>=32
Florfenicol	<=8	16	>=32
Tetracycline	<=4	8	>=16
Sulphamethoxazole	<=256	N/A	>=512

Table2: Prevalence of E. coli isolates resistant to one or more antibiotics at arrival

	Susceptible to all antibiotics tested	Resistant to one Antibiotic	Resistant to two antibiotics	Resistant to three or more antibiotics
% of Isolates at Arrival	84.6%	11.22%	1.98%	2.20%
Standard Deviation	0.0908	0.0755	0.0406	0.0338

Table 3: Proportion of isolates resistant to various antibiotics within treatment groups over 5 sampling times during the feeding period.

Antibiotic	Treatment Group	Arrival	Day 7	Day 15	Day 35	Pre-slaughter
Ampicillin	Control	0.694%	2.59%	2.78%	0.347%	4.51%
	Feed	2.08%	9.75%	10.39%	2.11%	8.11%
	Injectable	2.81%	4.17%	11.0%	4.31%	3.34%
Florfenicol	Control	2.78%^{a}	3.5%^{a}	9.03%	1.74%	20.11%
	Feed	9.72%^{ab}	20.39%^{b}	14.46%	6.41%	23.03%
	Injectable	3.85%^{b}	3.88%^{a}	10.26%	2.78%	17.33%
Sulpha-methoxazole	Control	3.13%	3.69%^{a}	5.21%^{a}	4.17%^{a}	15.34%
	Feed	7.29%	60.95%^{b}	50.63%^{b}	32.09%^{b}	20.60%
	Injectable	3.54%	8.36%^{a}	8.71%^{a}	8.4%^{a}	15.62%
Tetracycline	Control	6.94%	10.86%^{a}	18.4%^{a}	5.56%^{a}	34.22%
	Feed	12.85%	87.25%^{b}	93.21%^{b}	54.46%^{b}	40.13%
	Injectable	9.72%	22.19%^{a}	33.11%^{a}	17.78%^{a}	31.41%
Trimethoprim -Sulfa	Control	0.0%	0.347%^{a}	0.0%^{a}	0.0%	0.0%
	Feed	2.78%	12.03%^{b}	7.1%^{b}	1.77%	0.378%
	Injectable	0.726%	1.04%^{a}	0.726%^{a}	0.347%	0.0%

(*Treatment group means within time and antibiotic with different superscripts are significantly different at $p<0.05$)

Figure 1:

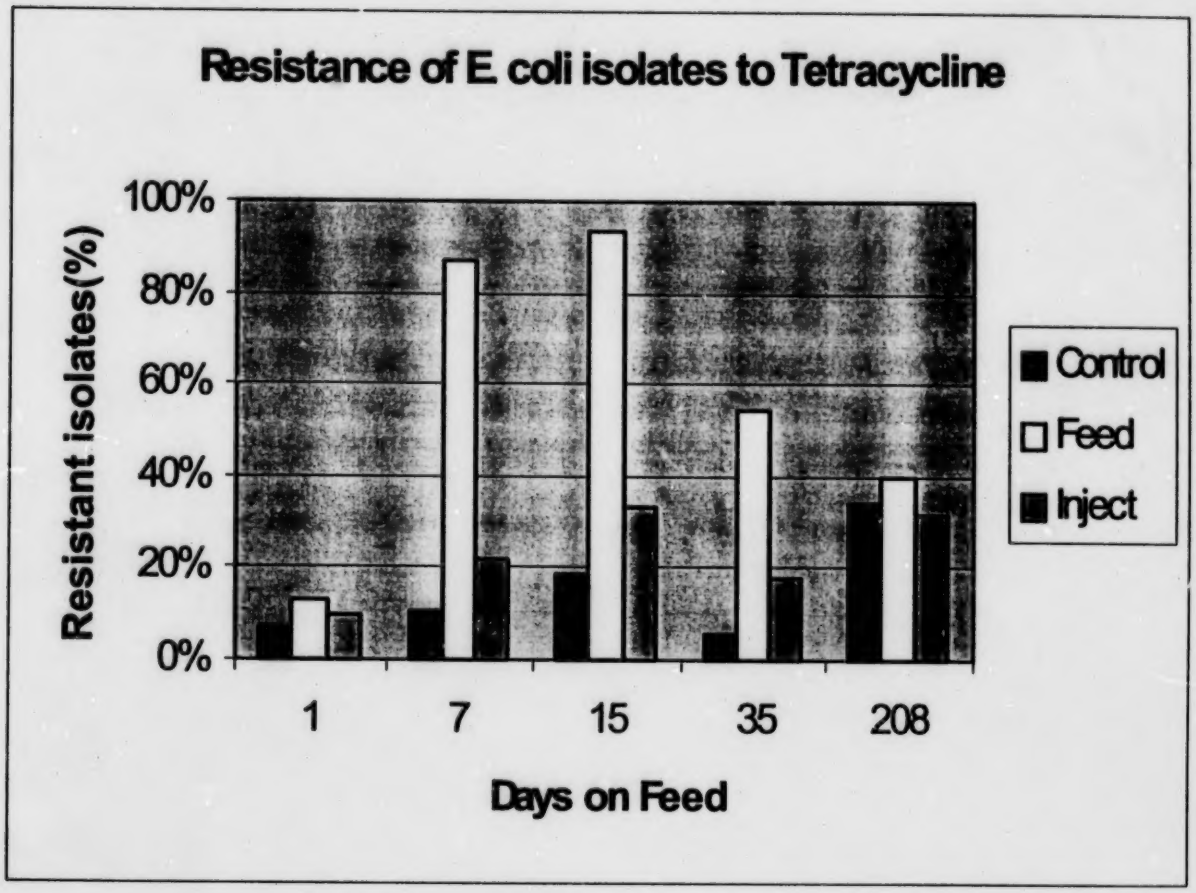

Resistance of E. coli isolates to Tetracycline
Resistant isolates(%)
100%
80%
60%
40%
20%
0%
1
7
15
35
208
Days on Feed
Control
Feed
Inject

Figure 2:

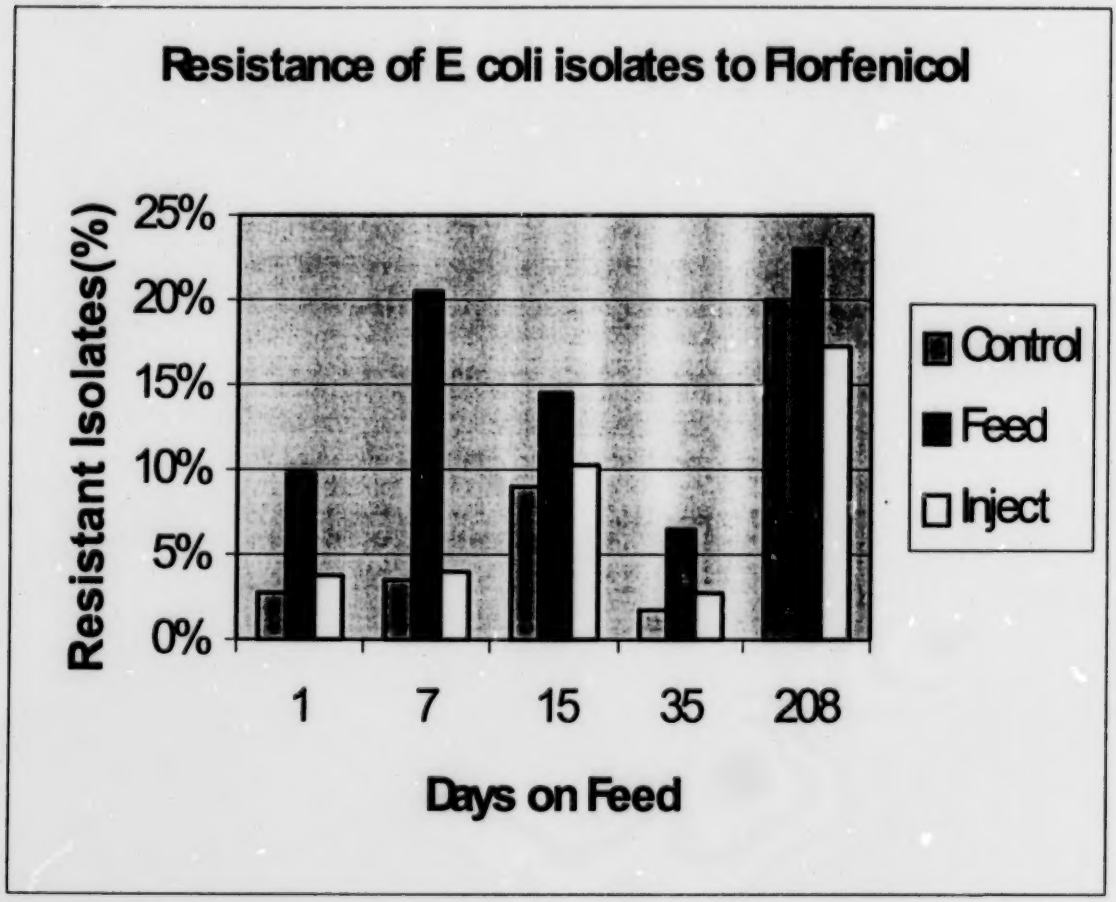

Resistance of E coli isolates to Florfenicol
Resistant Isolates(%)
25%
20%
15%
10%
5%
0%
1
7
15
35
208
Days on Feed
Control
Feed
Inject

Figure 3:

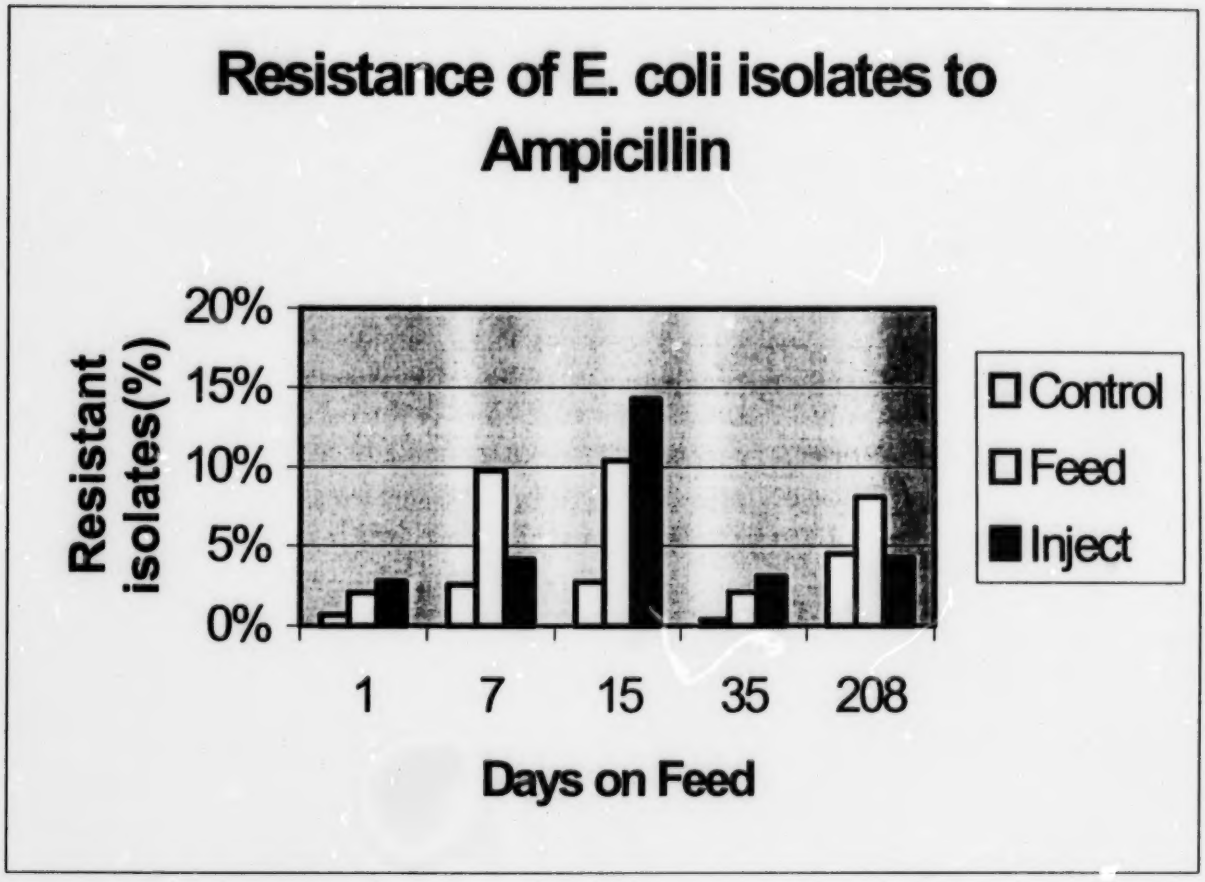

Resistance of E. coli isolates to Ampicillin
Resistant isolates(%)
20%
15%
10%
5%
0%
1
7
15
35
208
Days on Feed
Control
Feed
Inject

Figure 4:

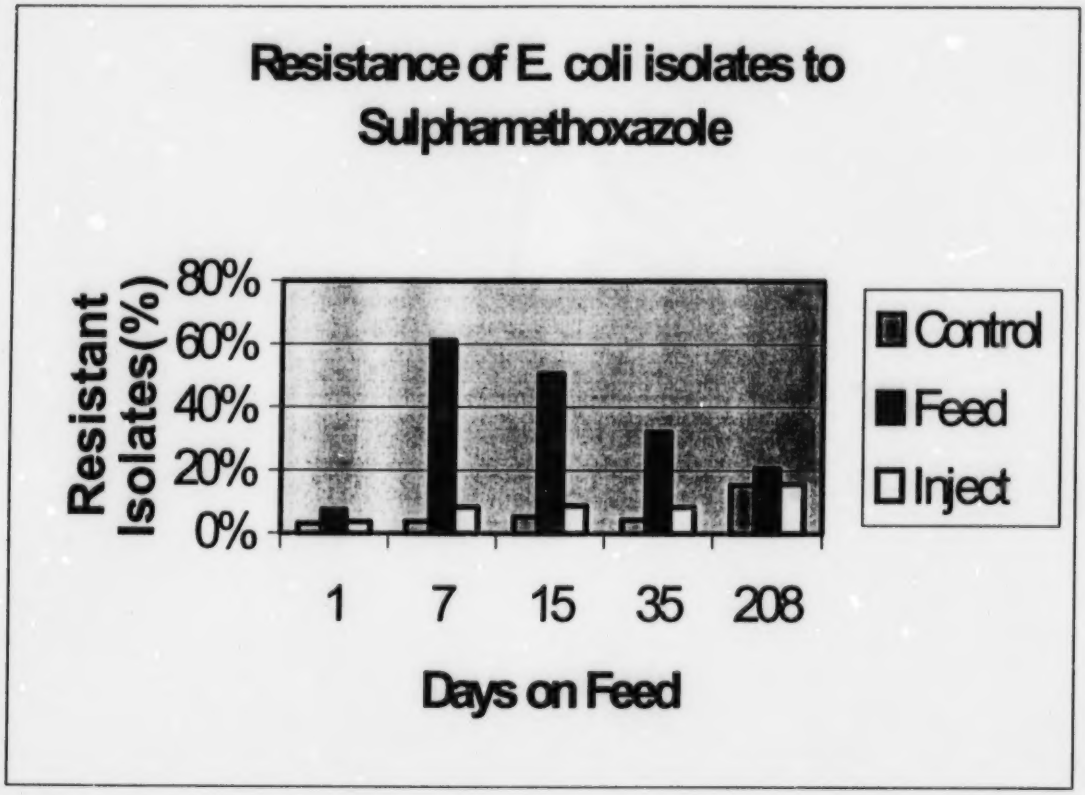
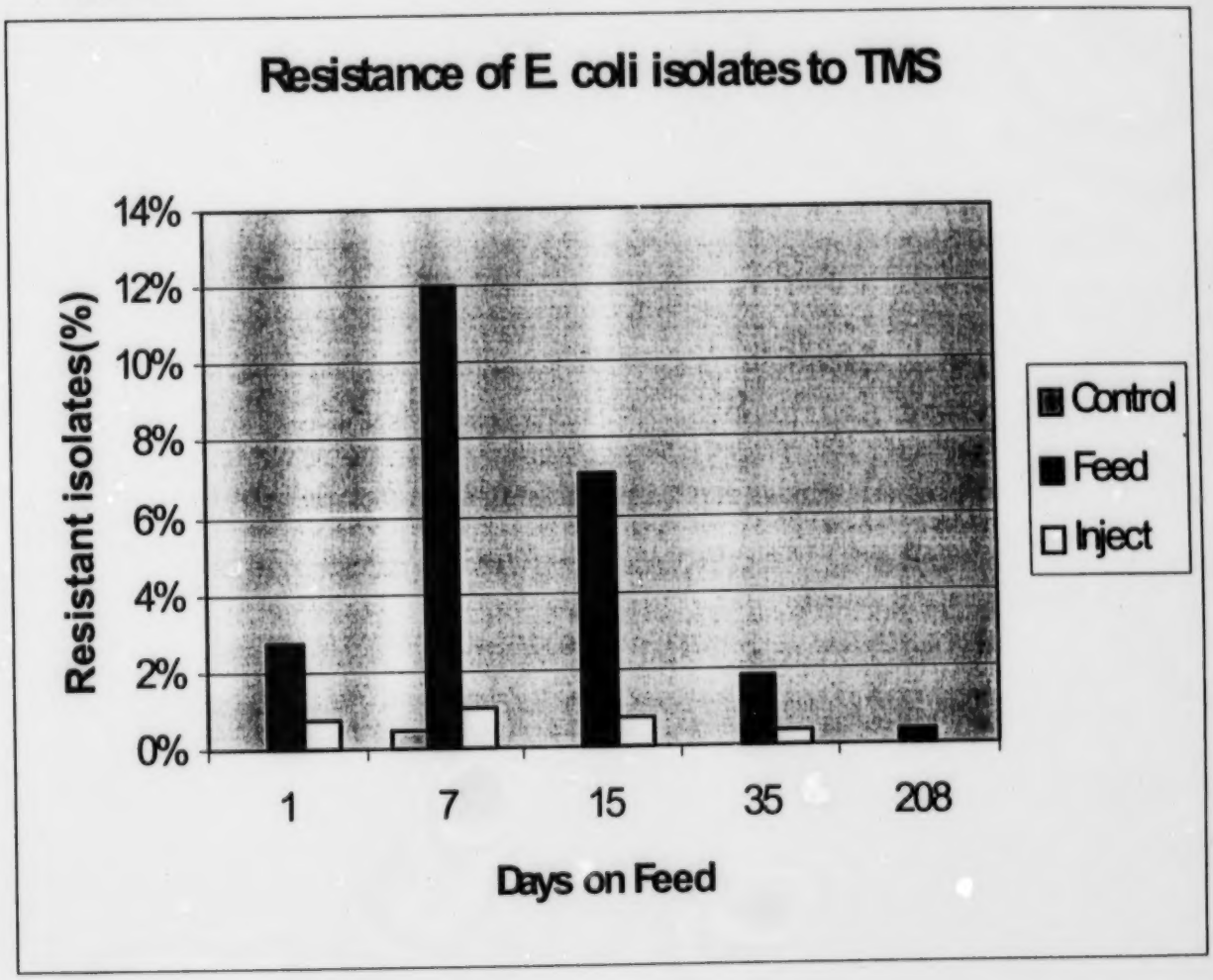

Resistance of E. coli isolates to Sulphamethoxazole
Resistant Isolates(%)
80%
60%
40%
20%
0%
1
7
15
35
208
Days on Feed
Control
Feed
Inject

Figure 5:


Resistance of E. coli isolates to TMS
Resistant isolates(%)
14%
12%
10%
8%
6%
4%
2%
0%
1
7
15
35
208
Days on Feed
Control
Feed
Inject